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Poplar growth and wood production on a grassland irrigated for decades with potato starch wastewater

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Abstract Irrigation of grasslands with potato starch wastewater causes changes in soil quality parameters, often resulting in decreased crop yields and sometimes causing animal diseases. Reduced agricultural income leads to designation of such grasslands for afforestation aimed at production of bioenergy crops and improvement of soil quality. In this study, *Populus alba* L. and seven different poplar clones were planted in 2009 on the grassland irrigated in 1973–2008 with potato starch wastewater and in non-irrigated, experimental forest. The survival, growth and wood production potential of planted poplars after 2–3 growing seasons were analyzed. Morphophysiological parameters of leaves, nutrients in leaves and fine roots, sugars in fine roots, leaf rust resistance, frost hardiness and their effects on growth were also determined. Stem diameter and stem volume index of poplars growing on the grassland were higher than in the experimental forest. Only *Populus deltoides* Bartr. × *P. maximowiczii* Henry clone 'Eridano' (in short ERI) had a survival rate of 100 % and the highest values of stem diameter (77 mm) and stem volume index (0.034 m^3) in comparison with other poplars in both sites after 3 growing seasons. Clone ERI was also characterized by a high leaf rust resistance and frost hardiness. The results suggest that clone ERI is suitable as a bioenergy crop on grasslands irrigated with potato starch wastewater. Afforestation of the grassland improved the fertility of the soil by increasing concentrations of soil organic matter and availability of mineral nutrients (N, P, K, S and Fe).

Keywords *Populus* spp. · Wastewater · Marginal and degraded land · Land-use change

Introduction

Fruit and vegetable processing industry, wineries, distilleries as well as olive oil and starch production use huge volumes of water and generate large quantities of wastewater (Hamilton et al. 2011; Kretschmer et al. 2002). Globally, production of olive mill wastewater itself attains about 7–30 million × 10^3 L per year (Mateo and Maicas 2015). The potato processing industry produces 8–28 × 10^3 L of wastewater per every 1000 kg of raw potatoes (Charmley et al. 2006). Wastewater reuse in agriculture irrigation is a common practice (Aryal and Reinhold 2015; Buelow et al. 2015). About 20 million ha of land worldwide are irrigated with wastewater (Hamilton et al. 2011).

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Annual production of starch amounts to 72 million tones in a global scale and approximately 10 million tones in the European Union (EU) (The European Starch Industry Association 2014). In the EU, starch potatoes make 31 % of agricultural raw materials and the share of potato starch in the total starch production is about 20 % (The European Starch Industry Association 2014). In industrial starch production from potato (*Solanum tuberosum* L.), huge amounts of potato starch wastewater (PSW) are derived from potato washing, rinsing and starch processing. PSW is rich in organic substances, inorganic suspensions and macronutrients (Arienzo et al. 2009). Because of the high concentrations of organic substances and nutrients, attempts were made to use PSW as substrate for production of microorganisms (Chang et al. 2008; Vu et al. 2010) and electricity (Lu et al. 2009). However, most often PSW is used to irrigate and fertilize fields (Rosenwinkel et al. 2005).

Unfortunately, PSW application for many years leads to unfavorable changes in nutrient content of the soil. The wastewater usually includes high concentrations of N (1200 mg L^{-1}) and K (1800 mg L^{-1}) (Arienzo et al. 2009). Potassium from PSW may be immediately available for plants, so irrigation with PSW may also lead to increasing the total and bioavailable concentrations of K in soil (Arienzo et al. 2009). However, this surplus K concentration may induce Mg deficiency in soil (Römheld and Kirkby 2010) and a decrease in Mg, Ca, Zn, Al and Mo levels in plants (Öborn et al. 2005).

Potatoes, legumes and grasses are K accumulators, but grasses accumulate K mainly in leaves (Arienzo et al. 2009). Fodder production and grazing from grassland after long-term irrigation with PSW may lead to various animal diseases and lower growth rate of livestock (Öborn et al. 2005). At the time of increasing competition, the suboptimal productivity and insufficient finances for balanced fertilization of arable land and grassland lead to their exclusion from agricultural use and designation for afforestation aimed at biomass production for energy (Broeckx et al. 2012; Dillen et al. 2013).

Poplars are some of the most promising woody species that can be grown in environments of varying fertility, with relative tolerance to biotic and abiotic stress conditions and high biomass and stem-wood production. In Europe and North America, high yields of biomass from rural areas were obtained for poplar clones *Populus* × *interamericana* Brockh clones 'Hoogvorst' (in short HOO), 'Beaupré' (in short BEA) and 'Boelare' (in short BOE) (Dillen et al. 2013; Johansson and Karačić 2011; Truax et al. 2012), *Populus* × *euramericana* (Dode) Guinier clone 'Robusta' (in short ROB) (Broeckx et al. 2012; Kačík et al. 2012; Verlinden et al. 2013), *Populus maximowiczii* × *P. trichocarpa* Torr. et Gray clone 'Hybrid 275' (also known as 'NE 42', in short NE) (Baum et al. 2012; Gruenewald et al. 2007), *Populus alba* clone 'Villafranca' (in short VIL) (Kačík et al. 2012; Rosso et al. 2013), and *Populus deltoides* Bartr. × *P. maximowiczii* Henry clone 'Eridano' (in short ERI) (Coyle et al. 2006a, b; Kaczmarek et al. 2013). Therefore our working hypothesis was that these poplar clones can be also suitable for growth on grassland irrigated for a long time with PSW.

Biomass production depends on poplar clones' ability to cope with climatic conditions and diseases (Coyle et al. 2006b; Zalesny et al. 2009). For example, frost, drought, or leaf rust infection can distinctly lower yields (Aylott et al. 2008; Coyle et al. 2006b. Poplar clones HOO, BEA, BOE, ROB, NE, VIL and ERI grow in cutting orchards and in an old poplar plantation of our Institute of Dendrology (ID PAS). Therefore, their susceptibility to leaf rust and extreme weather conditions sometimes occurring in Poland was known to us.

The first objective of the present study was to determine which poplar clones are the most productive on the grassland irrigated with PSW. Therefore we evaluated the survival rate of selected poplar clones, their height, stem diameter and volume. Secondly, we investigated the effect of frost hardiness, leaf rust resistance as well as leaf and root morphophysiological characteristics on poplar growth and biomass production. We planted rooted cuttings, as differences in rooting ability of poplars may also affect their growth potential (Zalesny Jr and Bauer 2007). Our third objective was to investigate the effect of poplar planting on soil quality related to soil mineral concentrations and enzymatic activities (phosphomonoesterases) important in the metabolic capacity of the soil and for nutrient cycling (Nannipieri et al. 2012). Afforestation of agricultural land leads to an increase, decrease or no change in soil organic matter, carbon and nitrogen content in the first few years, depending on soil quality, soil management and species of tree planted (Laganière et al. 2010). Poplars are fast-growing trees and their extensive root system as well

as abundant leaf fall improve the soil structure and increase nutrient cycling (Truax et al. 2012). Finally, the field experiment with different poplar clones for 3 growing seasons (GS1, GS2, and GS3) on the grassland irrigated for decades with PSW is compared with a parallel experiment in a non-irrigated, experimental forest (where other poplars were grown previously). The two sites differ in soil texture, pH and concentrations of essential mineral elements. We discuss the suitability of selected poplar clones for survival, biotic and abiotic tolerance, growth and wood production potential in both environments as well as for revitalization of the grassland soil irrigated with PSW.

Materials and methods

Site description

The potato processing plant in Trzemeszno (52°33′N, 17°49′E) was established about 130 years ago and since 1973 the industrial wastewater after treatment has been sprayed onto 170 ha of grassland and about 500 ha of agricultural land. In the last 3 years before our study, according to data from the processing plant, it generated ca. 3400 m^3 of PSW per day, with pH varying from 4.71 to 5.86, biological oxygen demand (BOD_5) ranging from 2015 to 3777 mg O_2 dm^{-3}, relatively high concentrations (mg dm^{-3}) of N (515–751), P (128–361), K (1613–2222) and antifoaming agents in excess. Irrigation with PSW was repeated many times (about 90 days per year), usually from September to December.

Approximately 0.55 ha of grassland irrigated for decades with PSW (hereinafter referred to as grassland) was allotted for the study and the last sprinkling irrigation of this area with PSW was 4 months before its planting with poplars. The grassland was dominated by perennial ryegrass (*Lolium perenne* L.), white clover (*Trifolium repens* L.), cocksfoot (*Dactylis glomerata* L.) and in some places by couch grass (*Elymus repens* (L.) Gould). Prior to poplar planting, the grassland was disked. In adjacent grassland, there were no areas non-irrigated with PSW that could be used as reference fields. Therefore a similar plot to the grassland was established in the experimental forest (hereinafter referred to as forest) of ID PAS in Kórnik (52°14′N, 17°05′E). The forest site was formerly used for cultivation of poplar trees of other varieties than those used in the present study, for about 20 years. Those trees were harvested for lumber, plywood, pallets and fuel in 2006. The site was then ploughed and disked, and white mustard was grown there. After 2 years, the mustard biomass was ploughed into the soil as green manure.

Climatic data were obtained from weather stations nearest to the study sites, i.e. in Kórnik (52°14′N 17°05′E) for forest and in Gniezno (52°31′N 17°36′E) for grassland. During the study period (2009–2011), mean annual temperatures ranged from 7.9 to 9.7 °C in Kórnik and from 6.9 to 8.8 °C in Gniezno (Fig. 1 in supplementary material). Annual precipitation was 577 mm in 2009, 751 mm in 2010 and 451 mm in 2011 in Kórnik, compared to 603 mm in 2009, 709 mm in 2010 and 427 mm in 2011 in Gniezno.

Soil analysis

Soil samples were collected at 16 points on each site from the depth of 0–25 cm (without undecomposed plant and animal parts) immediately before poplar planting in March 2009. Soil pH and concentrations of N_{Total} (organic and N–NH_4), C_{Total}, S_{Total}, P_{Total}, bioavailable forms of N (N–NH_4 and N–NO_3), S (S–SO_4), P (P–PO_4) as well as total and bioavailable (EDTA) forms of metals (K, Mg, Ca, Fe, Cu, Cr, Zn, Cd and Pb) were determined exactly as previously described (Lorenc-Plucińska et al. 2013). Each soil sample was analyzed separately (with 3 replications). Pooled samples for each site (6 replications) were used to determine soil texture and total organic matter (OM) as in our earlier study (Lorenc-Plucińska et al. 2013), while bulk density, electrical conductivity and cation exchange capacity as reported by Kalra and Maynard (1991).

The forest site was postagricultural land with sandy loam texture, alkaline pH and a low bulk density (Table 1). The concentrations of OM were high, like N_{Total} and C_{Total}, which resulted in a low C:N ratio. Concentrations of P_{Total}, K_{Total} and S_{Total} were in the normal range for forest soil. However, plant-available phosphorus (P–PO_4) was scanty, indicating P deficiency. There was a very high concentration of Ca_{Total} (4.1 %), and thereby of Mg_{Total}. High concentrations of Ca_{Total} resulted in high cation exchange capacity and electrical conductivity, dependent on cation exchange capacity. In a short distance from the experimental area, the soil contains marl and probably calcium carbonate was spread there.

The grassland soil had mainly sandy loam and sandy textures, irregularly arranged (in 70 and 30 % of the

Table 1 Physicochemical characteristics of the forest and grassland soil before poplar planting (March 2009) and after the last growing season (GS3, November 2011)

Characteristic	March 2009		November 2011		p values[a]	
	Forest	Grassland	Forest	Grassland	Forest	Grassland
Texture (% sand:silt:clay)	75:25:0	84:16:0				
pH (H_2O)	7.74 (0.18)	6.2 (0.4)**	7.59 (0.02)	5.4 (0.2)**	0.007	<0.0001
pH (1 n KCl)	7.37 (0.04)	4.9 (0.2)**	7.27 (0.02)	3.9 (0.4)**	<0.0001	<0.0001
Specific gravity (kg dm^3)	1.13 (0.04	1.38 (0.06)**				
EC($\mu S\ cm^{-1}$)[b]	112 (10)	56 (8)**				
CEC (cmol kg^{-1})[c]	16.4 (0.4)	2.3 (0.4)**				
Organic matter (%)	5.2 (0.8)	1.84 (0.32)**	7.3 (1)	2.4 (0.2)**	<0.0001	0.0005
C_{Total} (%)	3.3 (0.6)	0.9 (0.2)**	4.4 (0.4)	1.3 (0.06)**	0.001	<0.0001
C:N	12.3 (1.6)	7.8 (1)**	14.1 (0.6)	10.1 (0.6)**	0.012	0.0002
N_{Total} (%)	0.27 (0.04)	0.12 (0.04)**	0.31 (0.02)	0.13 (0.02)**	0.023	ns
N–NH_4 (mg kg^{-1})	1.9 (0.6)	1.5 (0.6)	1.9 (0.2)	2.4 (0.2)**	ns	ns
N–NO_3 (mg kg^{-1})	9 (2)	5 (1)*	12.4 (2)	33 (2)**	ns	<0.0001
P_{Total} (mg kg^{-1})	539 (60)	635 (96)	511 (78)	1152 (144)**	ns	<0.0001
P–PO_4 (mg kg^{-1})	0.9 (0.6)	11 (4)**	1.9 (0.2)	25.9 (1.4)**	0.003	<0.0001
K_{Total} (mg kg^{-1})	546 (86)	1771 (84)**	657 (36)	1696 (92)**	0.041	ns
K–EDTA (mg kg^{-1})	28 (16)	286 (20)**	44 (6)	386 (20)**	0.031	<0.0001
Mg_{Total} (mg kg^{-1})	1134 (162)	742 (90)**	1095 (46)	810 (72)**	ns	ns
Mg–EDTA (mg kg^{-1})	68 (10)	34 (6)**	64 (4)	36.5 (1.6)**	ns	ns
Ca_{Total} (mg kg^{-1})	41,026 (7436)	1077 (190)**	29,657 (2418)	582 (34)**	0.001	<0.001
Ca–EDTA (mg kg^{-1})	6626 (678)	952 (130)**	7084 (1375)	511 (54)**	ns	<0.0001
S_{Total} (mg kg^{-1})	375 (80)	170 (26)*	217 (20)	102 (30)**	ns	0.021
S–SO_4 (mg kg^{-1})	2.1 (0.8)	1.8 (0.4)	17.9 (1.8)	11.1 (1)**	< 0.0001	<0.0001
Fe_{Total} (mg kg^{-1})	5910 (1390)	3894 (220)*	6348 (852)	4229 (306)**	ns	ns
Fe–EDTA (mg kg^{-1})	34 (6)	64 (16)**	41.9 (1.6)	124 (10)**	ns	<0.0001
Cu_{Total} (mg kg^{-1}	8.1 (0.8)	6.7 (1)				
Cu–EDTA (mg kg^{-1}	0.78 (0.12)	0.99 (0.04)				
Cr_{Total} (mg kg^{-1})	20 (4)	19 (2)				
Cr–EDTA (mg kg^{-1})	0.68 (0.04)	0.15 (0.04)**				
Zn_{Total} (mg kg^{-1})	30 (4)	22 (2)*				
Zn–EDTA (mg kg^{-1})	3.3 (1.0)	3.9 (0.8)				
Cd_{Total} (mg kg^{-1})	0.26 (0.04)	0.32 (0.06)				
Cd–EDTA (mg kg^{-1})	0.108 (0.02)	0.06 (0.04)*				
Pb_{Total} (mg kg^{-1})	15 (2)	5 (2)**				
Pb–EDTA (mg kg^{-1})	1.2 (0.4)	0.91 (0.18)				

Mean and 2 SE values of 16 independent soil samples collected in 2009 and 45 and 41 independent soil samples collected in 2011 from grassland and forest, respectively. Significance of differences between samples from forest and grassland: * $p < 0.05$; ** $p < 0.01$

[a] Significance of differences between samples from 2009 and 2011: p values, ns = not significant

[b] Electrical conductivity

[c] Cation exchange capacity

tested samples, respectively), slightly acidic, and had a low cation exchange capacity (Table 1). The concentrations of OM were low, which could be a result of the application of the PWS, which probably also contributed to the relatively high specific gravity of soil. The N_{Total} content was quite low, proportional to the amount of C_{Total}, which gave a narrow range of C:N ratio. The concentration of $N–NH_4$ was normal but $N–NO_3$ level was low. Total phosphorus concentrations were within the normal range, but the concentration of $P–PO_4$ was rather low. Total and bioavailable forms of Ca and Mg_{Total} concentrations were at the lower limit of standards for agricultural soils (Mocek and Drzymała 2010). In contrast, concentrations of both forms of potassium were higher than usual in fields and the K ions determined the cation exchange capacity, although it usually depends on Ca ions. In both sites, soil concentrations of Cu, Cr, Zn, Cd and Pb were within the normal range, but we detected an Fe deficit on the grassland (Kabata-Pendias and Pendias 2001).

Soil analysis was also performed after poplar planting. The fraction of soil adhering to roots of each poplar (rhizosphere) in both sites was collected in November of GS3, simultaneously with the harvest of fine roots. The fresh soil samples were split into two parts: one air-dried at room temperature for elemental analysis, and the other kept moist at −4 °C in a climate chamber for enzymatic analysis. The first part was used to determine soil pH and concentrations of C and C:N ratio, as well as total and bioavailable N, P, S, K, Mg, Ca and Fe. Each soil sample was analyzed in triplicate for element measurements and with 4 replications for the activities of alkaline phosphomonoesterase (EC 3.1.3.1, Alkali–Pase) and acid phosphomonoesterase (EC 3.1.3.2, Acid–Pase). Element concentrations and the Acid–Pase and Alkali–Pase activities were analyzed exactly as earlier described by Lorenc-Plucińska et al. (2013).

Plant material

The native European white poplar (*Populus alba* L., and poplar clones (HOO, BEA BOE, ROB, NE, VIL and ERI) were planted in the forest and grassland in March 2009. The plants derived from dormant hardwood cuttings (20–30 cm long) collected from one-year-old stems on stool beds established at cutting orchards of the ID PAS. The cuttings were rooted in the forest from March 2008 to February 2009. All new shoots except one were removed as they appeared. In early March 2009, the rooted cuttings with single stems of similar height were transplanted onto the grassland or to a new place in the forest. They were planted manually with a shovel at the depth of 35–45 cm. Within each site, each poplar clone was represented by 15 trees. Randomized block design was used, with 3 blocks of 8 poplar species or clones and 5 trees of each clone or species per plot, in 4 single rows (tree spacing: 4 m × 7 m, i.e. 358 trees ha^{-1}). Both sites were fenced to protect the young trees against deer browsing. During the study, poplars in both sites were not irrigated with PSW or water, and no supplemental nutrients were added to the soil. In May 2009 and 2011, glyphosate (a systemic herbicide) was applied around each planted poplar, protected by a covering during the treatment. Once a year the understory between the rows was mown to suppress plant competition. In winter of each year, tree branches were pruned in order to shape the main stem and the tree top, as the crop was to be the single-stem system in 10–15 years. Trees that died in April of the first growing season (GS1) were replaced with new planting, but the replacement trees were not included in the study.

Leaves: chlorophyll, specific leaf area (SLA) and nutrient concentrations

Ten leaves from different crown positions of trees were used for non-destructive chlorophyll measurements with SPAD–502 Minolta (Japan) chlorophyll meter in mid-June of GS3. Three SPAD readings were taken on each leaf and then averaged per leaf. After the readings, 5 leaves were randomly selected and served as one pooled sample for chlorophyll extraction. The other 5 leaves were used for nutrient analysis. Chlorophyll $a + b$ concentrations were analyzed according to Lichtenthaler and Wellburn (1983). In order to convert SPAD values into absolute units of chlorophyll $a + b$ concentrations, calibration curves were derived for each clone and site. Concentrations of C, N, P, K, Ca, Mg and Fe in leaves were determined exactly as in Lorenc-Plucińska et al. (2013). Each leaf sample (pooled from 5 leaves) was analyzed in triplicate.

Right after chlorophyll estimation, additional 10 leaves from different crown positions of trees were collected for morphometric measurements. Projected leaf area (PLA) of scanned leaves (Epson Perfection V700 Photo, Model: J221A, Seiko Epson Corp., Japan) was measured with the software WinFOLIA Pro (Regent Instruments Inc. Canada) and then they

were oven-dried at 70 °C for 72 h. The SLA was calculated by dividing the PLA by dry mass of the leaf.

Sunlit, healthy and non-senescent 10 leaves from different crown positions of poplars were also taken for nutrient analysis in mid-August of GS3. Leaves were randomly grouped into two pooled samples (each composed of 5 leaves) and analyzed in triplicate. Elemental analyses were carried out exactly as for the samples harvested in mid-June.

Leaves for chlorophyll, SLA and nutrient concentrations analysis were taken from randomly selected 9 trees for ERI, VIL, ROB and NE (i.e. 3 trees from each block × 3 blocks) on each site and 9 and 5 trees for HOO from grassland and forest, respectively. In the forest site, only 5 trees of HOO survived to GS3.

Fine roots: carbohydrate and nutrient concentrations

The fine root fraction (≤2 mm in diameter; in short: fine roots) from growing poplars was picked out by hand in November of GS3. Fine roots were taken from the same trees that were selected for leaf analysis. After collection, fine roots were manually separated from the soil and quickly and carefully washed in clean water. Living fine roots were separated from dead roots based on their lighter colour and greater resilience. Mycorrhizae associated with roots were regarded as part of the roots. Collected fine roots were analyzed for carbohydrates with 4 replicates, while for element concentrations in triplicate. Soluble carbohydrates and starch were extracted and determined using a standard colorimetric assay exactly as described in Szuba et al. (2013) and expressed as percentage of dry mass. Elemental analyses were carried out exactly as for leaf samples harvested in mid-June.

Analysis of survival, resistance to frost damage and leaf rust, tree growth and stem volume

Survival rate (% of living trees out of the initially planted cuttings) was recorded in April of GS1, GS2 and GS3. Resistance of poplars to frost damage was assessed in March of 2011, because of the very hard winter of 2010/2011, and to late spring frost in May of GS3, because of unusually low temperature on 4 and 5 May 2011 (−4.5 and −2.7 °C, respectively) (Fig. 1). In both cases, damage was rated on a scale of 0–3, where 0 = no damage of the apical buds by frost and young leaves by spring frost, and 3 = severe (>75 %) terminal mortality by frost and blackened leaves by spring frost.

Resistance of poplars to leaf rust (*Melampsora* spp.) was assessed in September of GS2 and GS3. The incidence of rust was recorded for 30 leaves from each crown position by visual assessment using a scale of 0–4, where: 0 = no incidence; 1 = up to 25 % of leaf area infected; 2 = 26–50 % of leaf area infected; 3 = 51–75 % of leaf area infected; and 4 = 76–100 % of leaf area infected. Assessment of winter and spring damages and rust incidence was done for all growing poplars in both sites.

Height and stem diameter at breast height (1.3 m, in short DBH) of all growing trees were measured in December of GS2 and GS3. Tree height was measured with a pole, while diameter, with a digital caliper. To assess wood productivity, potential stem volume index (hereinafter referred to as stem volume) was calculated as tree height × DBH^2 (m^3).

Statistical analysis

Data were analyzed using STATISTICA 10 (StatSoft Inc., USA). All results are presented as mean values with standard error (±SE). Means were compared by using Fisher's least significant difference (LSD) test. Differences were considered significant at $p < 0.05$. Two-way analysis of variance (ANOVA) was used to examine the significance of factors: poplar clones and sites and their interaction. For each site, linear correlations between leaf characteristics were estimated with Pearson's correlation coefficient on a clone mean basis.

Results

Leaves: chlorophyll, SLA and nutrient concentrations

SPAD readings were closely correlated with chlorophyll concentration in the leaves of trees growing in the forest ($r = 0.96$; $p < 0.0001$) and grassland ($r = 0.78$; $p < 0.0001$). Chlorophyll concentrations in the leaves from the grassland were higher than in those from the forest ($p < 0.0001$). On both sites, clone VIL had the highest chlorophyll concentration in leaves and clone ERI had the lowest (Fig. 1).

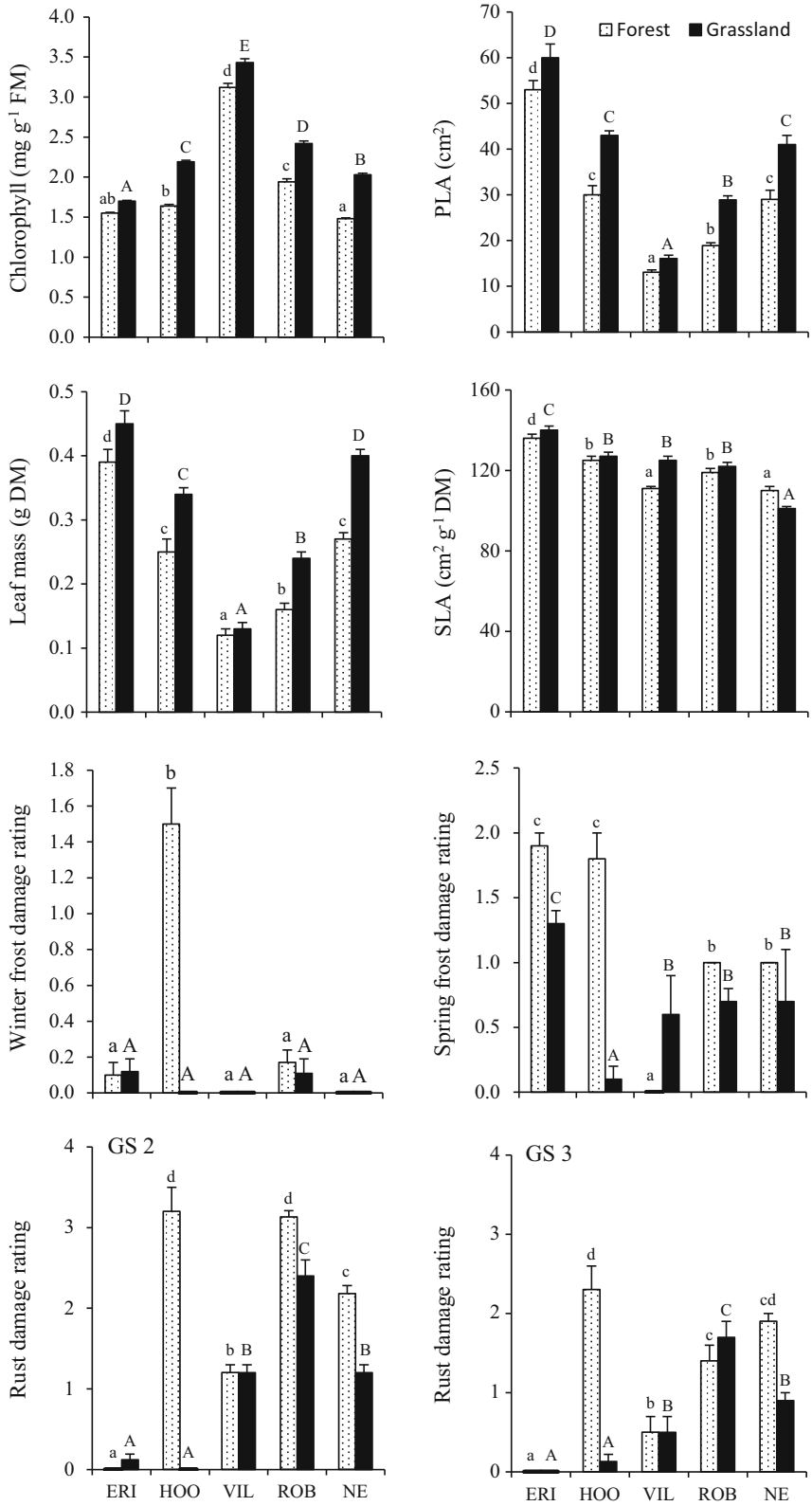

Fig. 1 Total chlorophyll concentration in leaves, projected leaf area (PLA), leaf dry mass, specific leaf area (SLA), as well as winter and spring frost and leaf rust damage in poplars from the forest and grassland. Measurements of chlorophyll, PLA, leaf dry mass, and SLA were carried out in June of the third growing season (GS3). 10 leaves were randomly sampled, from 9 trees of ERI, VIL, ROB and NE clones in both grassland and forest, and for HOO from 9 and 5 trees respectively in the grassland and forest. Winter and spring frost damage was assessed in March and May of GS3, for 15 trees of ERI and VIL, 5 of HOO, 12 of ROB, and 11 of NE clone from the forest, as well as for 15 trees of ERI and HOO, 11 of VIL and ROB, and 10 of NE clone from the grassland. Leaf rust damage of poplars was assessed in September of the second growing season (GS2) and GS3. In GS2, 30 leaves were randomly sampled, from 15 trees of ERI, HOO, VIL, and ROB and 14 of NE clone in the forest, as well as from 15 trees of ERI, HOO and VIL, 11 of ROB and 14 of NE clone in the grassland. In GS3, numbers of trees were the same as in the assessment of winter and spring frost damage. The results are presented as means with one standard error. Significant differences between data are indicated with different letters (LSD test, $p < 0.05$). FM and DM: fresh and dry mass, respectively

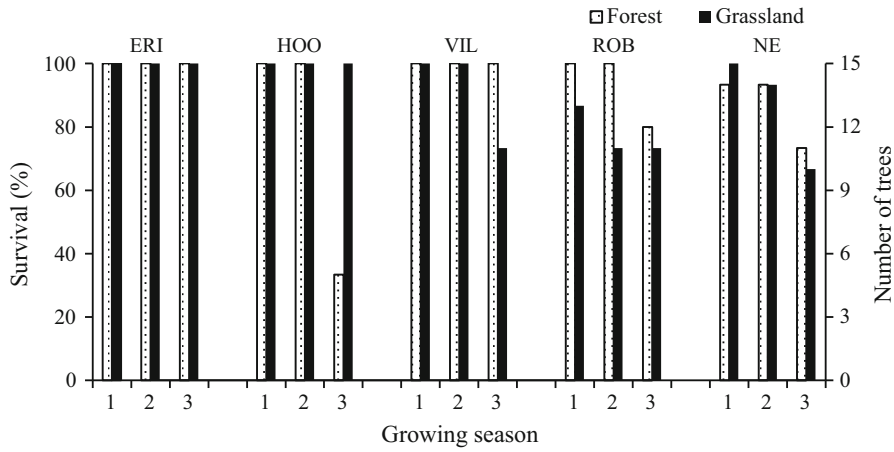

Fig. 2 Survival rate among poplar clones (ERI, HOO, VIL, ROB and NE) and number of tress in the forest and grassland after 1–3 growing seasons (GS1, GS2 and GS3)

The leaves of poplar clones growing in the two sites differed in PLA and dry mass (grassland > forest, $p < 0.0001$), while SLA was similar (grassland ≈ forest, $p > 0.05$). Leaves of ERI and NE had the highest and lowest SLA, respectively (Fig. 1). Leaves of ERI were also the heaviest, while VIL leaves were the lightest.

Younger leaves of all poplar clones from the grassland had higher concentrations of N, K and Fe, and lower values of Ca, Mg (except VIL) and P (except ERI and HOO) and C:N ratio, as compared with those from the forest (Table 1 in supplementary material). Older leaves of poplar from grassland were also characterized by higher concentrations of N, K, Mg and P and lower values of Ca content and C:N ratio (Table 1 in supplementary material). Concentrations of C and Fe were similar in leaves from both sites.

Fine roots: carbohydrate and nutrient concentrations

On the grassland, about 90 % of fine root biomass of planted poplar clones was distributed almost directly under the forest litter. This value was particularly high in ERI. In the forest site, nearly 80 % of fine roots were found at 10–15 cm soil depth. Concentrations of soluble carbohydrates (SC), starch and total non-structural carbohydrates (i.e. the sum of SC and starch; TNC) were lower in fine roots of poplar clones from the grassland than in those from forest ($p < 0.0001$ for SC; $p = 0.004$ for starch and $p < 0.0001$ for TNC) (Fig. 2 in supplementary material). In fine roots of poplar clones from grassland, concentrations of C, N, P, K, and Mg were higher, while Ca and Fe content and values of C:N ratio were lower than in those from the forest ($p < 0.0001$ for all elements) (Table 2 in supplementary material).

Survival, resistance to frost damage and leaf rust, tree growth and stem volume

Survival rate of planted poplar clones (i.e. ERI, HOO, VIL, ROB and NE) was variable (Fig. 2) but the site effect on clone survival after GS1, GS2 and GS3 was not significant (forest ≈ grassland, $p \geq 0.05$). All planted cuttings of ERI survived in both sites. HOO showed 100 % survival rate on the grassland but very low (33 %) in the forest. All specimens of VIL survived in the forest, while on the grassland site strong wind broke trunks of 4 trees weakened by small poplar borer (*Saperda populnea* L.), resulting in decreased survival rate (73 %) after GS3. Clones ROB and NE had survival rates of 80 and 73 % in the forest and 73 and 67 % on the grassland, respectively (Fig. 2). During the planting, *P. alba*, BOE and BEA had no signs of diseases or bud frost damages. Nevertheless, all specimens of BOE died already in April of GS1, whereas BEA and *P. alba* had very low (13–20 %) survival rates (data not shown). Due to the low survival rates these poplars were not included in the subsequent measurements.

We recorded very slight winter frost damage of all poplar clones growing in both sites with the exception of HOO from the forest (Fig. 1). In contrast, there were strong late spring frost damages, mainly of ERI in both plantations and of HOO in the forest (Fig. 1).

Leaf rust infection scores differed between GS2 and GS3 (Fig. 1). Leaves of HOO from forest were the most infected by rust, whereas leaves of VIL were infected to a similar extent in both sites. On the other hand, ERI in both plantations and HOO on grassland were only very slightly infected by leaf rust.

The differences in mean tree height between the grassland and forest (Fig. 3) were negligible (forest $\approx$ grassland, $p \geq 0.05$). However, in ANOVA the effects of clone and site as well as clone $\times$ site interaction on tree height were significant ($p = 0.006$) after GS3 (Table 2). DBH and stem volume of trees growing on the grassland was higher than in the forest ($p < 0.0001$ and $p < 0.001$, respectively) and the effect of clone and site on both was evident (Fig. 3; Table 2). The larger DBH of poplar clones growing on the grassland in comparison with the forest was more pronounced after GS3 than after GS2, with the exception of VIL, which reached similar values in both sites after GS3 ($p > 0.05$). The same applied to stem volume (Fig. 3; Table 2).

Soil element concentrations and enzymatic activity after GS3

In comparison to the rhizosphere in the forest, the rhizosphere soil in the grassland had lower pH and concentrations of C_{Total}, N_{Total}, Ca_{Total}, Ca–EDTA, Mg_{Total}, Mg–EDTA, S_{Total}, S–SO_4, Fe and C:N ratio but higher concentrations of N–NO_3, N–NH_4, P_{Total}, P–PO_4, K_{Total}, K–EDTA and Fe–EDTA in November of GS3 (Table 1). When pH and essential mineral elements were compared before poplar planting (in March 2009) and after GS3 (November 2011) then the direction of changes in pH and C_{Total}, P–PO_4, K–EDTA, Ca_{Total}, S–SO_4 concentrations in the forest and grassland were similar but different for N_{Total}, N–NO_3, N–NH_4, K_{Total}, S_{Total} and Fe–EDTA (Table 1). The pH in the rhizosphere of individual poplar clones on the grassland was lower than in the forest (Table 3). Differences were also noted in concentration of all the analyzed elements, with the exception of the C:N ratio for VIL, the concentration of N–NH_4 for VIL, ROB and NE, and Mg–EDTA and S–SO_4 for NE, which were similar in both sites (Table 3). Activity of Acid–Pase was higher ($p < 0.0001$) and Alkali–Pase was lower ($p < 0.0001$) in the rhizosphere soil of the grassland than in the forest after GS3 (Fig. 3 in supplementary material; Table 2).

Discussion

Morphophysiological characteristics of leaves and fine roots

It is assumed that PLA, SLA and N concentration are leaf characteristics linked to net assimilation rate, tree growth and productivity (Broeckx et al. 2012; Johansson and Karacic 2011). PLA is often independent, while SLA and leaf N levels are dependent on environmental conditions and genotypes (Al Afas et al. 2007; Marron et al. 2007). In our study, PLA, SLA and N concentrations in young leaves varied between sites and clones, and the highest values were achieved by the best growing clone ERI (Figs. 1, 3; Table 2, and Table 1 in supplementary material). SLA was correlated with N in leaves of trees on the grassland ($r = 0.70$; $p < 0.0001$) and in the forest ($r = 0.37$; $p = 0.019$). N concentrations in young and mature leaves ranged from 2.5 to 3.4 % on the grassland and from 1.9 to 2.7 % in the forest (Table 1 in supplementary material), i.e. were within the range reported for *Populus* leaves (Pellis et al. 2004). Although N was a non-limiting factor on both plantations, the significantly lower N level in leaves in the forest than on the grassland ($p < 0.0001$ for young leaves and $p = 0.005$ for mature leaves) may reflect their lower photosynthetic capacity, depending on the N concentration (Al Afas et al. 2007; Weih and Rönnberg-Wästljung 2007).

Lower chlorophyll concentrations in leaves from forest than from grassland ($p < 0.0001$) (Fig. 1) can also indicate their lower photosynthetic capacity. Highly significant correlations were found between chlorophyll and leaf N and Fe concentrations in the forest ($r = 0.86$; $p < 0.0001$ and $r = 0.78$; $p < 0.0001$, respectively), earlier also reported in other studies (Lombard et al. 2010; Weih and Rönnberg-Wästljung 2007). Chlorophyll concentrations in leaves from the grassland were correlated with leaf Fe and Mg content ($r = 0.88$; $p < 0.0001$ and $r = 90$; $p < 0.0001$, respectively) but not with leaf N content ($r = 0.27$; $p > 0.05$). Mg and Fe play crucial roles in the chlorophyll molecule and photosynthesis, and SPAD readings were closely related to levels of these foliar elements (Klooster et al. 2012; Lombard et al. 2010). The unreliable relationship between leaf chlorophyll and N concentrations on the grassland may be a consequence of the impact of N

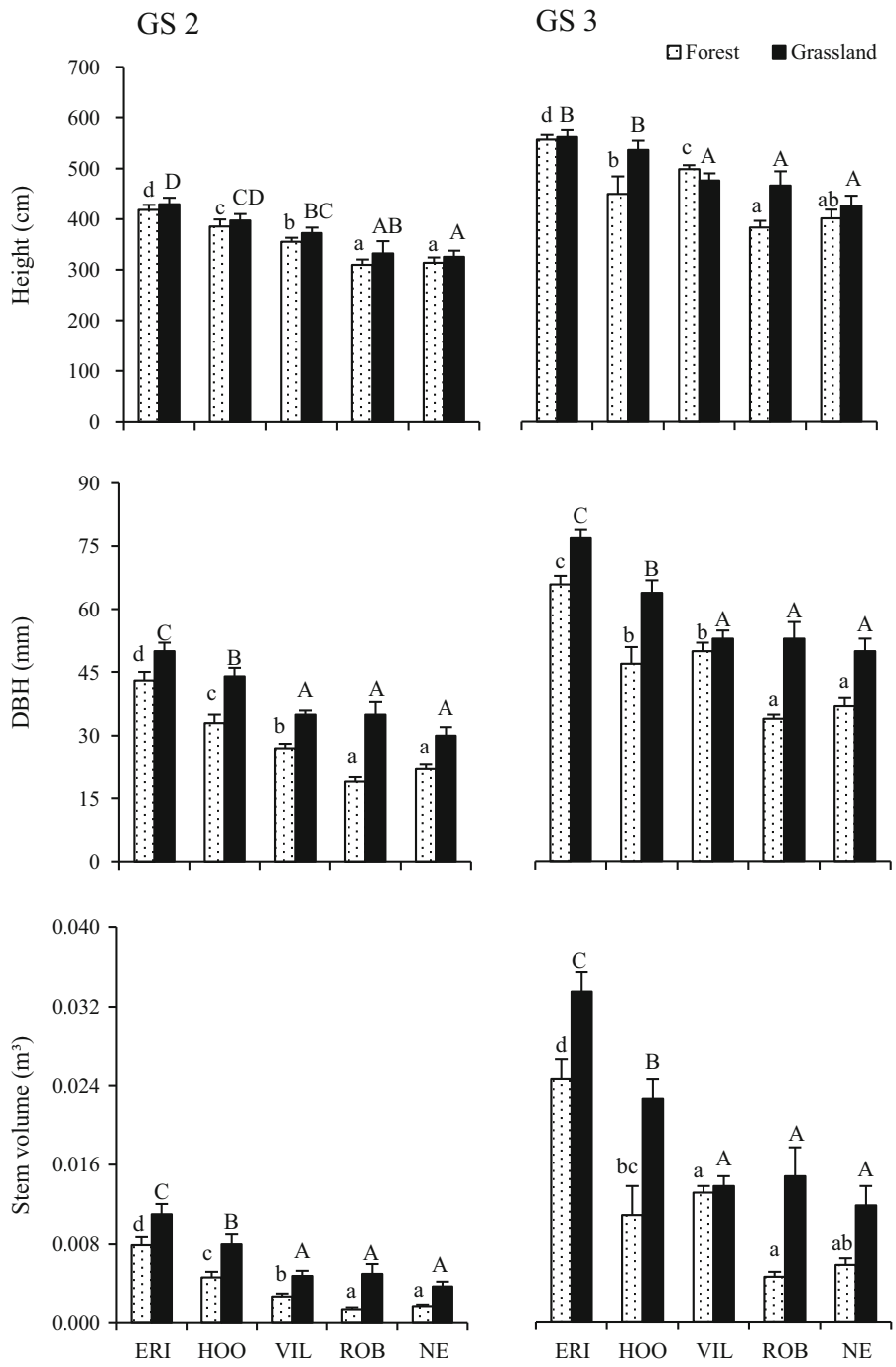

Fig. 3 Tree height, diameter at breast height (130 cm, DBH) and stem volume after second and third growing seasons (GS2 and GS3) for poplars in the forest and grassland. Other explanations as in Fig. 2

concentration and distribution within the leaf and also other factors on SPAD readings (Klooster et al. 2012; Lombard et al. 2010). The lowest chlorophyll concentrations were noted for clone ERI (Fig. 1), which showed the best growth parameters (Fig. 3). Carbon concentrations in leaves were similar for all poplar clones at both locations (Table 1 in supplementary material). This confirms earlier suggestions about the lack of relationship between chlorophyll and C content and tree growth (Marron et al. 2007).

Table 2 Significance of differences in response variables between poplar clones (ERI, HOO, VIL, ROB, and NE) from forest and grassland

Variables	ERI	HOO	VIL	ROB	NE	ANOVA effects (p values)[a]		
						Clone (C)	Site (S)	C × S
Total chlorophyll	**	**	**	**	**	<0.0001	<0.0001	<0.0001
Projected leaf area	*	**	**	**	**	<0.0001	<0.0001	<0.0001
Leaf dry mass	ns	**	ns	**	**	<0.0001	<0.0001	<0.0001
Specific leaf area	ns	ns	**	ns	**	<0.0001	0.007	<0.0001
Soluble carbohydrates	*	ns	**	**	**	<0.0001	<0.0001	<0.0001
Starch	**	ns	**	**	**	<0.0001	<0.0001	<0.0001
Total non-structural carbohydrates	**	ns	**	**	**	<0.0001	<0.0001	<0.0001
Winter frost damage	ns	**	ns	ns	ns	<0.0001	<0.0001	<0.0001
Spring frost damage	**	**	ns	**	ns	<0.0001	<0.0001	<0.0001
Rust leaf damage (in GS 2)	ns	**	ns	*	**	<0.0001	<0.0001	<0.0001
Rust leaf damage (in GS 3)	ns	**	ns	ns	**	<0.0001	<0.0001	<0.0001
Tree height (after GS 2)	ns	ns	ns	ns	ns	<0.0001	ns	ns
Tree height (after GS 3)	ns	*	ns	*	ns	<0.0001	0.002	0.006
Tree diameter at breast height (after GS 2)	*	**	**	**	**	<0.0001	<0.0001	ns
Tree diameter at breast height (after GS 3)	**	**	ns	**	**	<0.0001	<0.0001	0.012
Stem volume (after GS2)	*	**	**	**	*	<0.0001	<0.0001	ns
Stem volume (after GS3)	**	*	ns	**	**	<0.0001	<0.0001	0.025
Activity of acid phosphomonoesterases	**	**	**	**	**	0.017	<0.0001	<0.0001
Activity of alkaline phosphomonoesterases	**	**	**	**	**	<0.0001	<0.0001	<0.0001

Significance levels: * $p < 0.05$; ** $p < 0.01$; *ns* indicates $p \geq 0.05$

[a] Results of ANOVA for effects of poplar clone (C), site (S) and C × S interaction

On the grassland, about 90 % of fine roots of planted poplar clones were found in the top soil (0–5 cm soil depth; excluding the leaf litter) and the value was particularly high for ERI clone. In the forest site, nearly 80 % of fine roots were at 10–15 cm soil depth, like in other studies with deciduous trees (Terzaghi et al. 2013). The surface location of fine roots on the grassland increases their access to rainwater and nutrients from litter fall. This may result in higher concentrations of N and Mg in fine roots on the grassland than in the forest (Table 2 in supplementary material) despite the lower N and Mg concentrations in the soil on the grassland (Tables 1, 3). On the other hand, the fine roots in the top soil on the grassland could be vulnerable to pathogenic bacteria, fungi and insects living in/on the leaf litter. However, in the course of the study, there was no visible damage increase on fine roots from the grassland in comparison with the forest. Further, high K (Table 2 in supplementary material) and low soluble sugars concentrations in the fine roots from the grassland (Fig. 2 in supplementary material) may also indicate that fine roots were not attractive to pathogens and insects (Amtmann et al. 2008; Römheld and Kirkby 2010). The growth of fine roots in the top soil may lead to frost damage in case of severe winter, especially without snowfall. During our study, no such an event was observed.

Higher concentrations of N and C in fine roots from the grassland than from the forest were linked with lower values of the C:N ratio (Table 2 in supplementary material). The concentrations of C in fine roots are connected with their construction cost and N levels are connected with metabolic activity and root longevity, while the fine root C:N ratio can reflect their lifespan (Terzaghi et al. 2013). Assuming the above, our results seem to indicate a shorter lifespan and higher fine root turnover rate on the grassland than in the forest.

Table 3 Total concentrations of macro-and micronutrients and their bioavailable forms ($N–NO_3$, $N–NH_4$, $P–PO_4$, $S–SO_4$, K–EDTA, Ca–EDTA, Mg–EDTA, Fe–EDTA) and pH in the rhizosphere soil of poplar clones (ERI, HOO, VIL, ROB and NE) in the forest and grassland

Elements	Forest					Grassland				
	ERI	HOO	VIL	ROB	NE	ERI	HOO	VIL	ROB	NE
pH (H_20)	7.54 ± 0.01	7.58 ± 0.03	7.58 ± 0.01	7.60 ± 0.01	7.59 ± 0.03	5.26 ± 0.17	5.30 ± 0.05	5.42 ± 0.08	5.53 ± 0.08	5.51 ± 0.08
	a	ab	ab	b	ab	A	A	B	C	D
pH (1 n KCL)	7.23 ± 0.01	7.24 ± 0.02	7.24 ± 0.02	7.28 ± 0.02	7.29 ± 0.01	3.75 ± 0.23	3.88 ± 0.1	3.96 ± 0.04	3.96 ± 0.04	4.03 ± 0.07
	a	a	a	b	b	A	B	C	C	D
C_{Total} (%)	5.51 ± 0.08	3.85 ± 0.02	4.13 ± 0.08	4.3 ± 0.1	4.1 ± 0.1	1.41 ± 0.01	1.4 ± 0.1	1.19 ± 0.04	1.34 ± 0.04	1.18 ± 0.02
	c	a	b	b	b	C	BC	A	B	A
C:N	14.5 ± 0.4	14.1 ± 0.4	13.3 ± 0.7	13.2 ± 0.7	14.8 ± 0.4	9.5 ± 0.2	9.2 ± 0.4	**11.7 ± 0.4**	9.8 ± 0.3	10.5 ± 0.4
	ab	ab	a	a	b	A	A	C	A	B
N_{Total} (%)	0.37 ± 0.002	0.27 ± 0.002	0.31 ± 0.01	0.33 ± 0.01	0.28 ± 0.01	0.16 ± 0.001	0.15 ± 0.01	0.101 ± 0.001	0.14 ± 0.01	0.11 ± 0.004
	c	a	b	b	a	C	BC	A	B	A
$N–NH_4$ (mg kg^{-1})	1.8 ± 0.1	1.5 ± 0.1	1.7 ± 0.1	2.1 ± 0.1	1.7 ± 0.1	2.9 ± 0.1	2.6 ± 0.1	**2.1 ± 0.2**	**2.5 ± 0.2**	**2.2 ± 0.2**
	ab	a	a	b	a	B	AB	A	A	A
$N–NO_3$ (mg kg^{-1})	17.1 ± 0.3	8.2 ± 0.1	8.7 ± 0.3	15.9 ± 0.3	12.1 ± 0.3	42.3 ± 0.6	33.7 ± 0.8	26.3 ± 0.3	32.3 ± 0.2	30.5 ± 0.8
	d	a	a	c	b	D	C	A	C	B
P_{Total} (mg kg^{-1})	600 ± 50	390 ± 30	590 ± 70	520 ± 60	460 ± 40	1430 ± 100	1300 ± 80	1020 ± 80	1220 ± 70	730 ± 20
	b	a	b	ab	a	C	C	B	BC	A
$P–PO_4$ (mg kg^{-1})	2.4 ± 0.2	1.74 ± 0.1	1.8 ± 0.2	1.7 ± 0.1	1.3 ± 0.1	30 ± 1	27 ± 1	26 ± 1	25 ± 1	23 ± 1
	c	b	b	b	a	C	B	B	AB	A
K_{Total} (mg kg^{-1})	740 ± 50	620 ± 20	640 ± 10	710 ± 20	580 ± 20	1900 ± 30	1820 ± 40	1620 ± 40	1530 ± 40	1510 ± 20
	c	a	ab	bc	a	C	C	B	A	A
K–EDTA (mg kg^{-1})	53 ± 2	35 ± 1	60 ± 2	45 ± 1	32 ± 1	450 ± 20	370 ± 15	350 ± 14	310 ± 14	111 ± 20
	c	a	d	b	a	D	C	C	B	A
Mg_{Total} (mg kg^{-1})	1190 ± 30	1120 ± 60	1090 ± 80	1050 ± 20	1020 ± 10	1000 ± 20	890 ± 60	700 ± 30	725 ± 17	735 ± 20
	b	ab	ab	ab	a	B	B	A	A	A
Mg–EDTA (mg kg^{-1})	75 ± 2	56 ± 2	65 ± 2	72 ± 2	54 ± 6	40 ± 2	36 ± 2	39 ± 1	34 ± 1	**33 ± 2**
	c	a	b	bc	a	B	AB	B	A	A
Ca_{Total} (mg kg^{-1})	23,300 ± 900	30,000 ± 1000	30,000 ± 2000	34,000 ± 3000	30,600 ± 800	650 ± 20	610 ± 10	580 ± 20	520 ± 10	510 ± 20
	a	a	a	a	a	C	BC	B	A	A
Ca–EDTA (mg kg^{-1})	6300 ± 500	7600 ± 50	7400 ± 100	7500 ± 200	6600 ± 100	590 ± 20	556 ± 9	538 ± 10	490 ± 10	468 ± 20
	a	b	b	b	a	C	B	B	A	A

Table 3 continued

Elements	Forest					Grassland				
	ERI	HOO	VIL	ROB	NE	ERI	HOO	VIL	ROB	NE
S_{Total} (%)	0.03 ± 0.002	0.02 ± 0.001	0.02 ± 0.001	0.02 ± 0.001	0.02 ± 0.001	0.01 ± 0.0001	0.01 ± 0.0003	0.01 ± 0.0001	0.01 ± 0.0003	0.01 ± 0.001
	a	a	a	a	a	A	A	A	A	A
$S–SO_4$ (mg kg^{-1})	20.3 ± 0.7	17.1 ± 0.3	22.4 ± 0.3	17.4 ± 0.3	12.2 ± 0.3	13.9 ± 0.2	11.5 ± 0.1	9.4 ± 0.2	9.2 ± 0.2	**11.2 ± 0.2**
	c	b	d	b	a	B	B	A	A	B
Fe_{Total} (mg kg^{-1})	6300 ± 200	5400 ± 200	5700 ± 200	5600 ± 200	4800 ± 100	4900 ± 200	4700 ± 100	3800 ± 400	4100 ± 100	3600 ± 100
	c	b	b	b	a	C	BC	A	AB	A
Fe–EDTA (mg kg^{-1})	44 ± 1	43 ± 1	45 ± 3	39 ± 1	39 ± 1	148 ± 5	142 ± 5	118 ± 5	104 ± 4	107 ± 5
	b	b	b	a	a	C	C	B	A	AB

Mean values ± SE of 9 independent samples for ERI, VIL ROB, and NE clones from grassland and forest and 9 and 5 samples for HOO from grassland and forest, respectively, collected in November of the last growing season (GS3). Values designed with the same letters are not significantly different (LSD-test; $p \geq 0.05$). Bold font indicates lack of significant differences between the forest and grassland ($p \geq 0.05$)

Survival, frost hardiness, rust resistance, growth and stem-wood production

Of all the planted poplars, only clone ERI had the 100 % survival rate in both sites after GS3, which confirms its high ability to survive in various environments and growing conditions (Coyle et al. 2006a; Kaczmarek et al. 2013). In comparison with the other clones, leaves of ERI developed earlier in the spring and very quickly expanded the leaf blade, which became discolored and fell off in late autumn. However, a consequence of fast leaf development can be leaf damage due to late spring frosts. The sharp temperature drop in May after an early spring warm period (Fig. 1 in supplementary material) damaged expanding leaves of ERI, especially in the forest site (Fig. 1). However, damaged leaves were rapidly replaced with new ones, whose PLA, weight and SLA already in mid-June reached higher values than in the other poplar clones (Fig. 1). Clone ERI was also characterized by high leaf rust resistance and frost hardiness, like in earlier research (Coyle et al. 2006b. Clone ERI achieved the highest values of DBH and stem volume in comparison with other poplar clones after GS3 (Fig. 3). Stem volume for ERI (0.034 ± 0.002 m^3) in suboptimal growing conditions on the grassland was 2.7–5.0-fold greater after GS3 than for ERI in studies with fertilization and irrigation (Coyle et al. 2006a; Kaczmarek et al. 2013). Although these differences to some extent could be results of the growth environment, they clearly indicate the great potential for growth and stem-wood production of ERI clone on the grassland.

The HOO clone achieved good growth parameters on the grassland but it seemed inappropriate for growth in the forest (Fig. 3), where HOO was highly susceptible to *Melampsora* rust infection (Fig. 1). The consequences of leaf rust damage can include a decrease in photosynthetic efficiency and frost hardiness (Aylott et al. 2008), stunted growth and even mortality of young trees (Coyle et al. 2006b. Severe frost from 23 to 27 December 2010, when temperature dropped to −23.5 °C (Fig. 1 in supplementary material), killed most of the rust-infected trees. Also serious late spring frost damages were noted in rust-infected HOO trees in the forest (Fig. 1). Earlier reports indicate that the level of foliar rust damage depends on site conditions (Aylott et al. 2008), while others suggested that only certain poplar clones are

more susceptible to rust in a given area (Coyle et al. 2006b. Foliar rust damages of HOO as well as ROB and NE were generally higher in the forest than on the grassland (Fig. 1; Table 2). The sites greatly differed in soil conditions (Table 1) and the lower incidence of the disease on the grassland could be due to the high concentration of K in the soil, leaves and fine roots (Table 3 and Tables 1, 2 in supplementary material) of poplars growing there, as high K diminishes the incidence of diseases (Amtmann et al. 2008; Römheld and Kirkby 2010). A lower ability to survive as well as poorer growth were observed in VIL after GS3 (Fig. 3). That clone was resistant to frost and moderately susceptible to rust infection but significantly attacked by the insect pest *Saperda populnea* (L.). Curvature of the trunk of infested trees disturbed their growth performance, and the weakened trees were easily broken by the wind. VIL was previously recommended for wood plantations for energy (Kačík et al. 2012; Rosso et al. 2013), but considering our results, especially because of its susceptibility to *S. populnea*, it is not advised for use. Poplar clones ROB and NE are also often used for bioenergy plantations (Baum et al. 2012; Broeckx et al. 2012). However, results of this study show that ROB and NE rather should not be selected for stem-wood productivity under suboptimal soils, because of their damage by spring frost, low disease resistance and low growth parameters. The native European white poplar (*Populus alba*) and poplar clones BOE and BEA had also very poor survival rates (0–20 %, data not shown). On the other hand, these poplars grow well in the *Populetum* collection of ID PAS. The high survival rate and growth potential of *Populus alba*, BOE and BEA were the basis for their frequent applications in short-rotation coppice systems (Dillen et al. 2013; Johansson and Karačić 2011; Rosso et al. 2013; Truax et al. 2012). However, in our plantation, management of these poplars proved to be unsuitable for long-term stem-wood production.

Elemental analysis and enzymatic activity of the soil

Soil texture, pH and concentrations of essential mineral elements differed between forest soil and grassland before poplar planting. Differences remained also after 3 years of poplar growth, with the exception of concentrations of inorganic N (Table 1). Ammonium ($N–NH_4$) and nitrate ($N–NO_3$) concentrations were lower in grassland soil than in the forest before poplar planting and higher after 3 years of poplar growth in the grassland. This may indicate a rapid OM decomposition and nitrogen mineralization and nitrification (Stauffer et al. 2014) on the grassland after GS3, probably partly due to greater litter inputs through better growth of poplars and short lifespan and higher fine root turnover on the grassland than in the forest sites. The hypothesis of rapid OM biodegradation is also supported by a higher concentration of N in leaves (2.8 ± 0.1 % on the grassland and 2.4 ± 0.1 % in the forest; $p = 0.005$) and lower C:N ratios (20.8 ± 0.6 vs. 35.2 ± 0.8; $p < 0.0001$) in fine roots, which might effectively stimulate the growth of soil microorganisms (Mao et al. 2011).

The comparison of the chemical properties of forest soil before poplar planting and after GS3 indicates that poplar planting led to a reduction in alkaline pH and Ca_{Total} and an increase in OM, C_{Total}, N_{Total}, K_{Total}, $P–PO_4$, K–EDTA and $S–SO_4$ concentrations in rhizosphere soil after GS3 (Tables 1, 3). The greater N_{Total} concentration probably resulted from an increase in organic N, as the concentrations of inorganic N in the soil did not change after GS3. Plants can use organic N through enzymatic hydrolysis of proteins and peptides (Nacry et al. 2013) of soil OM, and its concentrations increased after GS3. A significant ($p = 0.023$) increase in the soil C:N ratio to ~ 14 after GS3 implies appropriate N availability in soil (Peichl et al. 2012). Moreover, concentrations of N_{Total}, $N–NH_4$ and $N–NO_3$ in the soil, as well as N in poplar leaves and roots (Tables 1, 2 in supplementary material) were within the ranges reported in the literature (Pellis et al. 2004). The very low concentrations of bioavailable P ($P–PO_4$) before poplar planting increased about 1.9-fold in rhizosphere soil after GS3, but without changes in P_{Total}. The latter might be due to the presence of stable, non-bioavailable forms of P in the high Ca_{Total} and alkaline soil pH (Shen et al. 2011). Despite the increase in the concentrations of inorganic P after GS3, the concentration of $P–PO_4$ in the rhizosphere can still be classified as very low. Nevertheless, the P concentrations in leaves and roots (Tables 1, 2 in supplementary material) were even higher than those given in the literature (Ciadamidaro et al. 2014). The increase in $P–PO_4$ in the forest after GS3 could be the result of the catalytic transformation of organic P

esters, due to a high activity of Alkali–Pase (303 ± 6 μg *p*–NP g^{-1} DM h^{-1}, Fig. 3 in supplementary material), effectively produced by soil bacteria, fungi and animals but not secreted by plant roots, and partly also of Acid–Phase (130 ± 3 μg *p*–NP g^{-1} DM h^{-1}, Fig. 3 in supplementary material), originating mainly from plant roots with rhizosphere microorganisms (Nannipieri et al. 2012).

Afforestation of the grassland led to a decrease in pH and Ca_{Total} and Ca–EDTA and an increase in OM, C_{Total}, P_{Total}, P–PO_4, K–EDTA, S–SO_4 and Fe–EDTA in the rhizosphere soil after GS3 (Table 1). No changes in N_{Total} and N–NH_4 were accompanied by a nearly sevenfold increase ($p < 0.0001$) in concentrations of N–NO_3. Nitrate is easily available as highly mobile anions in the top soil and may be taken up by plant roots. Poplars have a strong preference for N–NO_3-dominated soil solutions (Woolfolk and Friend 2003) at low soil pH (Lafleur et al. 2013). An about 1.5-fold increase ($p = 0.0002$) in the C:N ratio in the rhizosphere soil and its value near 10 after GS3, together with high nitrate concentrations, may indicate accelerated OM biodegradability as well as N mineralization and nitrification process, in which ammonium is converted to nitrate by nitrifying bacteria (Stauffer et al. 2014). Nitrification may acidify the soil (Colin-Belgrand et al. 2003). Therefore, it could be speculated that the decreased pH from 6.2 before poplar planting to 5.4 in the rhizosphere soil after GS3 (Table 1) may be partly attributed to increased nitrification. Soil pH could be also lowered by the release of acid exudates by plant roots and rhizosphere microorganisms (Jones 1998). Rhizosphere acidification might improve the mobilization and availability of soil elements (i.e. Ca, Mg, K, Fe and P) (Hinsinger et al. 2011; Lafleur et al. 2013) and thereby enhance nutrient uptake. The consequence of the latter may be the relatively high concentration of elements in the leaves and roots of planted poplar (Tables 1, 2 in supplementary material), similar to the values reported by others (Ciadamidaro et al. 2014; Kabata-Pendias and Pendias 2001), despite very low concentrations of e.g. Mg, Ca and Fe in the rhizosphere soil after GS3 (Tables 1, 2). The increase in bioavailability of nutrients could better satisfy the high nutrient demand of poplar for biomass growth, but at the same time could lead to depletion of base cations in soil (Lafleur et al. 2013), which was seen for Ca (Table 1), especially in the rhizosphere of ERI on grassland after GS3 (Table 3). In addition, the increased availability of P–PO_4 through dissolving P-minerals (Hinsinger et al. 2011) is probably not sufficient for growing poplar, as indicated by the high activity of Acid–Phase (221 ± 4 μg *p*–NP g^{-1} DM h^{-1}, Fig. 3 in supplementary material), hydrolyzing the soil organic P complexes (Amtmann et al. 2008; Nannipieri et al. 2012).

Conclusions

This study shows that clone ERI is the best candidate for potential stem-wood production among all the poplar clones planted in the forest and grassland irrigated for many decades with PSW. On the grassland the stem volume for ERI was 2.8-fold higher than in the slowest growing NE after GS3. It has been shown recently that for poplar growth, clonal selections can be made after GS3 (Kaczmarek et al. 2013). Therefore it can be assumed that clone ERI in a long-life plantation (10–15 years) will continue to have the highest stem volumes and thereby wood productivity of all the planted poplar clones in the forest and grassland. Climatic instability in the temperate climate of Poland (Central Europe) and resistance to leaf rust were important factors affecting survival and growth of poplar clones planted in the forest and grassland. The stronger leaf rust resistance of poplar planted in the grassland than in the forest and higher frost hardiness and less severe spring frost damage could have partly resulted from higher concentrations of K in the soil, leaves and fine roots of poplars growing in the grassland, and the above properties undoubtedly were responsible for the better growth of poplars planted in the grassland than in the forest. The beneficial effect of higher K in soil and crops on resistance to diseases and frost damage were already reported earlier (Amtmann et al. 2008; Römheld and Kirkby 2010).

Due to poplar planting on the grassland, soil quality parameters (OM, C_{Total}, N–NO_3, P_{total}, P–PO_4, K–EDTA, S–SO_4, Fe–EDTA concentrations) in the topsoil after GS3 were significantly better than before poplar planting and hence afforestation promoted revitalization of grassland soil. The increase in soil fertility can be a consequence of decomposition of high-quality leaves of the fast-growing poplars, but also of the short lifespan of fine roots and their high turnover rates. Accelerated litter decomposition,

together with increased soil C concentrations following poplar planting on the grassland, may indicate increased soil C sequestration after GS3. The nutrient enrichment of the soil under poplar was linked with soil acidification to pH 5.4, but it still remained in the optimum pH range (5.0–7.5) for poplar growth (Zalesny Jr and Bauer 2007). Simultaneously, Ca soil concentrations declined. Therefore there is a need for liming (preferably dolomitic lime) to promote further growth of the poplars and thus higher wood production on the grassland.

Land application of food processing wastewater represents its simple and cost-effective use (Kretschmer et al. 2002). However, as wastewater can be loaded with toxic trace elements or harmful organic substances, long-term application imposes a risk of groundwater contamination and threatens plant growth (Kretschmer et al. 2002). Aryal and Reinhold (2015) conclude that poplar trees meet the criteria of 'perfect' plants to be grown on fields irrigated with food processing wastewater and propose *Populus deltoides* × *P. nigra* DXN 34 poplars for planting on lands treated with fruit and vegetable processing wastewater. For improvement of soil fertility and bioenergy crops on lands irrigated with PSW, we suggest use of the ERI clone.

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